ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 8, Issue 6, 2015

ISSN - 0974-2441

Review Article

GENETIC AND ENVIRONMENTAL FACTORS INVOLVED IN HUMAN MALE INFERTILITY: A REVIEW

SHALAKA S RAMGIR, VALSALA GOPALAKRISHNAN ABILASH*

Division of Biomolecules and Genetics, School of Bio Sciences and Technology, VIT University, Vellore, Tamil Nadu, India.
Email: abilash.vg@vit.ac.in

Received: 06 August 2015, Revised and Accepted: 30 September 2015

ABSTRACT

As a World Health Organization (WHO) guidelines, infertility is the couple's inability to conceive after 2 years of regular unprotected intercourse. The investigation in male infertility is assuming greater importance because approximately half of all infertility cases caused by male factors. Although previous studies suggest that many cases with male infertility have a genetic and environmental etiology to the condition, and the majority of cases are idiopathic. About 10-20% of azoospermic patients are showing the microdeletion in Y-chromosome. In this deleted region, azoospermia factor (AZF) locus which is located in the Yq11 divided into the four regions as AZFa, AZFb, AZFc, and AZFd. In each of these regions a particular testicular histology and candidate genes have been found. The deleted in azoospermia gene family is also the most frequently deleted in AZFc region. Recently, not only Y chromosome, but X chromosome and some autosomal genes are also found in respect to male infertility. Frequent attacks on the naked mitochondrial DNA of sperm will responsible for oxidative damage or mutation to the mitochondrial genome and lead to male infertility. The introduction of molecular techniques, such as intracytoplasmic sperm injection, genomics, proteomics, metabolamics, has provided great perception into the genetics of infertility. Still our understanding to find a correlation between genotype and phenotype in male infertility remains limited.

Keywords: Infertility, Azoospermia factor, Deleted in azoospermia, Mitochondrial DNA, Intracytoplasmic sperm injection, Genomics, Proteomics, Metabolamics.

INTRODUCTION

The WHO announced infertility as the third most serious disease in the 21st century; where 48.5 million couples worldwide suffers from this reproductive system disease. Infertility is defined as couples are unable to conceive after a period of 2-year, but many couples look around after a year for medical suggestions [1]. As a American Urological Association, approximately 15% of couples are unable to conceive after 1 year of unprotected intercourse. A male factor is solely responsible in about 20% of infertile couples and contributory in another 30-40%. Male infertility is a multifactorial disorder. The genetic etiology includes chromosomal abnormalities, mitochondrial DNA mutations, and endocrine disorders of genetic origin. The non-genetic causes include hypogonadism, testicular maldescent, and structural abnormalities in the male reproductive tract, infection, impotence, chronic illness, medication, and immunological conditions. Moreover also numerous environmental factors are correlated with male infertility as temperature [2], noise associated with manufacturing [3] radiation exposure [4], electromagnetic waves [5], and a variety of chemicals [6]. Until now, various risk factors like temperature [7], automobile driving time a day [8], air pollution [9], regional differences in residential population density [10], mumps [11], stress [12], and alcoholism [13] are responsible for male infertility. Much research remains to be performed on the topic of male infertility, as many cases still receive "unknown cause" diagnosis. This present review focused on the genetic factors such as microdeletions on Yq, X-linked genes and autosomal genes and environmental factors involved in the context of human male infertility and also, molecular techniques used in assisted reproductive technology.

EPIDEMIOLOGY AND PREVALENCE OF MALE INFERTILITY

The distribution of infertility due to male factor ranged from 20% to 70% and that the percentage of infertile men ranged from 25% to 12% [14]. The major difficulties, which exist in the diagnosis of male reproductive dysfunction, serve to complicate the understanding of the epidemiology and a prevalence of male infertility [15]. Prospective epidemiologic studies may help physicians to understand male infertility [16].

AETIOLOGY OF MALE INFERTILITY

Infertility is either primary, where couples not become pregnant after at least 1 year of unprotected sex, or secondary where couples are able to get pregnant only for one time, afterward they are unable. Primary and secondary infertility is found in 67-71% and 29-33% of patients, respectively. Male factor is responsible for approximately 50% of the cases; where 30% are responsible for malefactors, and 20% are associated with male and female factors [1]. Male infertility has many causes from hormonal imbalances to physical problems, to psychological, and/or to behavioral problems. Moreover, fertility reflects a man's overall health [17]. About two-third population of infertile men have a problem with making sperm in the testes, either low numbers of sperm are produced and/or the sperm that are produced do not work properly. And also sperm transport problems are found in about one in every five infertile males. Obstructions in the tubes leading sperm away from the testes to the penis can cause a complete lack of sperm in the ejaculated semen [18]. The percentage of various causes for male infertility presented in (Fig. 1).

Molecular causes of male infertility are depend on spermatogenesis, which is a complicated process, and it is influenced by 2000 genes, most of them present on autosomes and approximately 30 genes are located on the Y chromosome. While autosomal genes regulate spermatogenesis and genes located on Y chromosome are not takes part in general body function except the male reproductive processes. In one survey of infertile men where sample size is 9766, the incidence of chromosomal abnormalities was 5% × 8% and of cases, sex chromosomal abnormalities are found in 4% × 2% of cases, and autosomal abnormalities are accounted for 1% × 5% [20]. In previous studies, it is observed that causes for male infertility 1-2% are occurred by pre-testicular (hypothalamus/pituitary) factors, 30-40% by testicular factors, 10-20% by post-testicular (obstruction) and 40-50% by non-classifiable [21]. List of various causes of male infertility presented in Table 1.

Table 1: Etiology for human male infertility [22-40]

Medical causes	Sperm defects, varicocele, infection, ejaculation issues, anti-sperm antibodies, tumors, undescended testicles, hormone imbalances, sperm duct defects, problems with sexual intercourse, celiac disease, certain medications, prior surgeries
Genetic causes	Chromosomal abnormalities, klinefelter syndrome, robertsonian translocation, Y chromosome microdeletions, AZFa, AZFb and AZFc deletion, partial AZF-c deletions. Cystic fibrosis, noonan syndrome, myotonic dystrophy, hemochromatosis, sickle cell disease, sex reversal syndrome, androgen receptor gene mutations, immotile cilia syndrome, mitochondrial deletions
Environmental causes	Industrial chemicals, heavy metal exposure, radiation or X-rays, overheating the testicles
Other causes	Illegal drug use, alcohol use, occupation, tobacco smoking, emotional stress, obesity, prolonged bicycling

AZF: Azoospermia factor

Table 2: Classification of male infertility according to semen parameters and sperm abnormalities

Abnormalities in sperm count	Definition
Oligospermia	A sperm count <20 million/mL
Azoospermia	Complete absence of sperms
Abnormalities in sperm motility	Definition
Asthenozoospermia	Sperm motility is <40%
Necrozoospermia	Non-viable sperm
Abnormalities in sperm morphology	Definition
Teratozoospermia	More than 40% of sperms are in abnormal form

MEDICAL CAUSES OF MALE INFERTILITY

Sperm defects

In many studies, it has been observed that male infertility is mostly due to sperm abnormalities. These abnormalities can be caused by congenital birth defects, disease, chemical exposure, lifestyle habits, and many other unknown factors. Classification of male infertility according to semen parameters and sperm abnormalities were presented in Table 2.

Azoospermia

It is present in 10-15% of infertile men. The main causes are a testicular failure and ductal obstruction [42]. Obstructive azoospermia: In this type of azoospermia; sperms are produced but not ejaculated due to a blockage in the sperm duct system. This condition observed in 7-51% of azoospermic men [43]. The most common reason is a vasectomy done to induce contraceptive sterility, [44] congenital (agenesis of the vas deferens as seen in cystic fibrosis), or acquired such as infection. Non-obstructive azoospermia - this type of azoospermia is due to failure of spermatogenesis within the testis. It is diagnosed in approximately 10% of infertile men [45]. It is caused by genetic factors, Y chromosome deletion (e.g. azoospermia factor [AZF]-c region), [46] karyotype abnormalities, radiation and toxins, medications, hormone imbalances, and varicocele. In one of the studies, it is observed that non-obstructive azoospermia is found in 10.7% of total population and obstructive azoospermia is found in 0.9% of cases [47]. Fig. 2 shows the percentage of various causes of infertility in couples and Fig. 3 represent the abnormal morphology of sperm.

Varicocele

Varicocele is defined as swelling of the veins that drain the testicle. It is the most common cause found in infertile males. This prevents the cooling of the testicle and leads to oligospermia and asthenozoospermia.

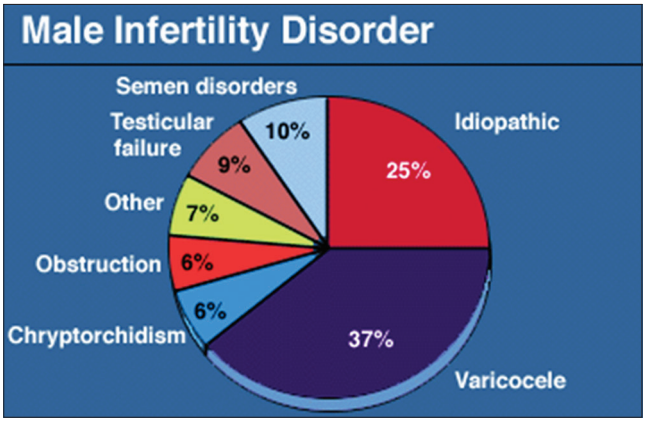

Fig. 1: The percentage of various causes of male infertility [19]

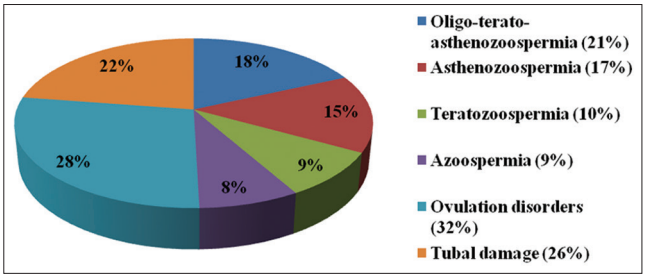

Fig. 2: The percentage of various causes of infertility in couples [41]

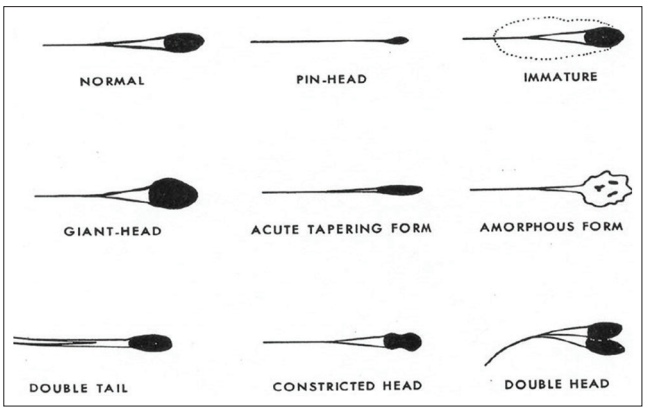

Fig. 3: Abnormal morphological forms of sperm [48]

Infection

Some infections are responsible to reduce sperm production or damage the sperm health, or it also blemishing that blocks the sperm passage. Infections, such as chlamydia, gonorrhea, prostatitis, mumps, orchitis, may result in permanent testicular damage.

Ejaculation problems

Retrograde ejaculation defined as when semen enters the bladder instead of emerging from the tip of the penis. Various health problems are responsible for this condition such as diabetes, spinal injuries, medications, and surgery of bladder, prostate or urethra.

Anti-sperm antibodies

These antibodies are immune system cells that mistakenly recognized sperm as harmful invaders and try to eliminate them.

Tumors

Malignant and nonmalignant tumors can affect the reproductive organs of male and also affects the pituitary gland. In some patients; surgery,

radiation, or chemotherapy for treatment of cancer can induce male fertility.

Undescended testicles
Some male patients, unable to descend their one or both testicles from the abdomen into the scrotum during fetal development and this cause decrease their fertility.

Hormone imbalances
Abnormalities in the endocrine system affect the male fertility. High levels of luteinizing hormone (LH) and follicle-stimulating hormone and low levels of gonadal steroids cause gonadal failure which is a cause of male infertility. High levels of LH cause hyper gonadotrophic hypogonadism (HH), which cause infertility.

Defects in vas deference: Illness or injury may damage the sperm ducts.

Sexual intercourse problems
Erectile dysfunction, premature ejaculation, and painful intercourse, abnormalities in structures of reproductive organs, such as hypospadias or psychological problems, may affect fertility.

Celiac disease
In this digestive disorder, patients are sensitive to gluten that is responsible for male infertility. After taking gluten-free diet, the male fertility may improved.

Prior surgeries
Vasectomy, inguinal hernia repairing, scrotal or testicular surgeries, prostate surgeries, large abdominal surgeries affect the sperm production.

GENETIC CAUSES OF MALE INFERTILITY [49]

Chromosomal aneuploidy
All studies investigating sperm aneuploidy levels in infertile men have demonstrated a three-fold increase in aneuploidy levels compared to their fertile males. Increases in sperm aneuploidy have been reported for all infertility phenotypes including oligozoospermia, asthenozoospermia, and teratozoospermia.

Chromosomal translocations
Nevertheless, carriers of balanced chromosomal translocations, while normal phenotypically, may experience reduced fertility, spontaneous abortions, or birth defects.

Robertsonian translocations
Robertsonian translocations involve chromosomes 13, 14, 15, 21, and 22. The results from various studies vary widely with reports of 3-36% unbalanced sperm.

Reciprocal translocations
Reciprocal translocations are seen in carriers, i.e. heterozygotes where studies have shown that 19-77% of spermatozoa are unbalanced.

Chromosomal inversions
As with chromosomal translocations, inversions can cause infertility, spontaneous abortions, and birth defects. In inversion carriers, it has been reported that unbalanced sperm ranges from 1% to 54%.

Numerical sex chromosomal abnormalities
Numerical sex chromosomal abnormalities in males are relatively common with Klinefelter syndrome (47, XXY) where the prevalence of 5% men with severe oligozoospermia and 10% with azoospermia. And in 47, XYY syndrome also sperm defects has been observed with a prevalence of 1-2 in 1000 live births; However, they are fertile.

GENETIC DISEASES (MONOGENIC AND MULTIFACTORIAL), WHICH ARE DIRECTLY OR INDIRECTLY ASSOCIATED WITH MALE INFERTILITY [17]

Cystic fibrosis
It is an inherited condition which involves the lungs and pancreas, but it also cause for infertility with or without mild sinus problems. The most men with cystic fibrosis suffer from obstructive azoospermia because they born without a vas deferens which causes male infertility.

Noonan syndrome
It is an inherited condition where males suffer from an abnormal gonadal (testicular) function which leads to infertility.

Myotonic dystrophy
It is an inherited multisystem condition with underdeveloped testes and abnormal sperm production which leads to male infertility in some cases.

Hemochromatosis
In this inherited condition, iron storage problem occurred. Moreover, 80% of males with hemochromatosis have testicular dysfunction.

Sickle cell disease
It is an inherited condition which affects the production of hemoglobin. In these patients gonadal failure, erectile dysfunction, and menstrual issues were found which lead to infertility [50].

Sex reversal syndrome
A man carrying a genetic sex chromosome of female leads to azoospermia and other characteristics related to infertility.

Androgen receptor (AR) gene mutations
A man with a defect in receptors for testosterone but showing normal male karyotype leads to infertility.

Immotile cilia syndrome
In this condition, sperms are nonmotile with a normal sperm count.

Other syndromes
Some syndromic cases associated with male infertility are Kartagener syndrome - Sperm immotility, metabolic syndrome [51], Persistent Mullerian duct syndrome, Aarskog–Scott syndrome, Kearns–Sayre syndrome, polyglandular failure syndrome (Types I and II), Bardet–Biedl syndrome, Prader–Willi syndrome, deafness–infertility syndrome [52], non-insulin dependent diabetes mellitus (hypogonadism, increased scrotal temperatures, impaired spermatogenesis, decreased sperm concentration and motility, and increased sperm DNA damage), dyslipidemia - increased oxidative stress (OS) in the testicular microenvironment and/or excurrent ductal system), Kearns–Sayre syndrome - cryptorchidism, pubertal delay, subnormal testicular volume, and low gonadotropin levels and others, which are yet to be investigated.

MICRODELETION OF THE Y CHROMOSOME

Microdeletions in the long arm of Y chromosome have been found at a much higher rate in infertile men than in fertile controls. Aberrations on the Y chromosome are currently found in approximately 15% of infertile males [53]. The high frequency of Y microdeletions suggests that the Y chromosome is susceptible to spontaneous loss of genetic material. 13% of azoospermic males, 1-7% of severely oligozoospermic males, 5% of males with severe primary testicular failure showed Y chromosome microdeletion [54]. Various studies focused on the "AZF locus," at Yq11 [1]. Gr/gr deletion in AZFc region is significantly associated with male infertility among Caucasians in Europe and the Western Pacific region [55]. Additional genes associated with spermatogenesis in men are RNA - binding motif protein (RBM), deleted in azoospermia (DAZ), SPGY, and testis specific protein; Y-linked (TSPY) which are

located on Y chromosome, and if deleted they reduce the chances of fertility [56]. The instability of the Y chromosome may be related to a high frequency of repetitive elements clustered along the length of the chromosome [57]. *De novo* deletions of Yq are the most frequent event in chromosomal abnormalities of men which are arise from recombinations of long stretches of highly repetitive DNA sequences in the process of meiosis or early pre-implantation development [58]. The introduction of intracytoplasmic sperm injection (ICSI) as an artificial reproduction technique may allow the transmission of Y deletion to the next generation [59].

MITOCHONDRIAL DNA MUTATIONS

Mitochondrial genome is producing many essential compounds of the respiratory chain which have a great impact on sperm motility. Sperm needs high adenosine triphosphate (ATP) to travel through female reproductive tract which accomplish by sperm mitochondria [49]. Mitochondrial genes as COX II, ATPase 6 and 8 plays an important role but after mutation in these genes ATP production disturbed and spermatogenesis and sperm motility is affected. Therefore, the mutations in mitochondial genome cause male infertility [60].

ENVIRONMENTAL CAUSES OF MALE INFERTILITY

Overexposure to certain environmental factors such as heat, chemicals, and toxins can reduce sperm production, and function. Specific factors include as following:

Industrial chemicals

High exposure to benzenes, toluene, xylene, pesticides, herbicides, organic solvents, painting materials, and lead may affect the sperm counts.

Heavy metal exposure

Lead or other heavy metals exhibition may cause male infertility.

Radiation or X-rays

Exposure to radiation may lower the sperm production, though it will often return to normal level, but if high doses of radiation given then sperm production may be permanently reduced.

Exposure of high temperature to testicles

Most commonly use of saunas or hot tubs can temporarily lower sperm count.

OTHER CAUSES

Illegal drug

To build up muscle strength and growth some anabolic steroids used which cause the testicles to shrink and to decrease sperm production. Cocaine or marijuana consumption also temporarily reduces the number and quality of sperms.

Alcohol

Drinking of alcohol may lower testosterone levels and also cause erectile dysfunction and lowers sperm production. Liver disease after taking of excessive alcohol may lead to fertility problems.

Smoking

Smokers may have a lower number of sperm count than non-smokers.

Emotional stress

Certain hormonal imbalances may occur by stress which is essential to sperm production. Severe emotional stress may lead to fertility problems such as, lower the sperm count.

Weight

Hormonal changes in obesity may reduce male fertility.

Prolonged bicycling

Prolonged cycling may reduce fertility due to overheating the testicles.

MOLECULAR ASPECTS IN MALE INFERTILITY

Structure of Y chromosome

The Y chromosome representing only 2-3% of the haploid genome, but it has around 107 genes and pseudogenes which has been mapped. Many of these are responsible for spermatogenesis and other male-related functions and deletion of any of these can result in infertility [61]. It has a high proportion of repetitive elements [62]. There is two pseduoautosomal regions, PAR1 on the short (Yp) and PAR2 on the long (Yq) arm of Y chromosome that recombine with their homologs on the X chromosome. The rest of the Y chromosome (~95%) is known as "non-recombining region" (NRY) or as "male-specific region." The NRY contains intrachromosomal repetitive elements that may be homologous to regions on X chromosome or Y chromosome [63]. The NRY region can be divided into euchromatic, centromeric and heterochromatic regions [64]. Y chromosome is divided into five broad sections - Pseudoautosomal boundary regions (PABY), Pericentric region on the short arm harboring the sex determining gene, euchromatic region (DYS1) cytogenetically known as Yq11 (subdivided into Yq11.21, Yq11.22, Yq11.23), is about 24 Mb on the proximal long arm, Heterochromatic Yq12 region (DYZ1, DYZ2) on the distal long arm contains 30 Mb and DYZ3 region that is critical for the survival and propagation of the Y chromosome [65]. Diagrammatic representation of genes and sequence tagged site (STS) markers present on Y chromosome is presented in Fig. 4.

Microdeletion on Y chromosome and male infertility

In humans, the Y chromosome is important for testicular differentiation and spermatogenesis. Recently, researchers found that observation of microdeletions on Yq11 in a high number of infertile men [67]. Previous studies found that interstitial microdeletions in Yq11 showed in 10-15% of idiopathic azoospermia and severe oligozoospermia, and this incidence is even higher in testicular diseases such as idiopathic Sertoli cell-only syndrome [68]. However in multiple non-overlapping spermatogenesis loci on Yq11 have been identified and that observed regions as AZFa, AZFb, and AZFc [69,70] including various genes essential for spermatogenesis. Later on, it is reported that the possibility of the presence of a fourth region, namely AZFd, between AZFb and AZFc. It has been reported that the deletion of a particular genes on AZF locus results in a characteristic phenotype because they are responsible for a particular stage in germ cell differentiation [71].

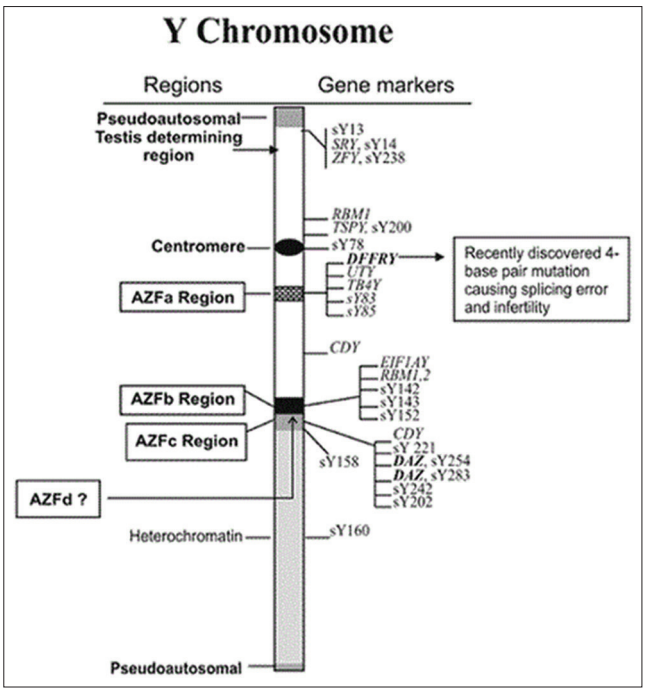

Fig. 4: Gene location and STS markers on Y chromosome [66]

CANDIDATE GENES IN AZFA REGION RESPONSIBLE FOR MALE INFERTILITY

DBY (DDX3Y)

DBY gene is located in the AZFa region, and it has important role in infertility because it is localized in the testis and is involved in the development of premeiotic germ cells [72]. In the study of the transcriptional activity of several AZF region genes found in men with Sertoli cell only syndrome (presence of sertoli cells in testes but a lack of sperms in the ejaculation [73] had reduced levels of DBY transcripts, but the other genes were transcribed normally. This finding suggests that DBY may play an important role in spermatogenesis, but further studies must be performed to replicate this result [74].

USP9Y

The USP9Y gene is also involved in spermatogenesis [75]. Shortening or deletion of USP9Y gene involved with azoospermia [76], oligozoospermia [77], or oligoasthenozoospermia [78]. However, it seems that this gene may involve in spermatogenesis process because it can be passed on to generations.

UTY

UTY is codes for a protein which is rich in tetratricopeptide repeats which might be include in the protein- protein interactions. The gene was mapped to the 5C interval corresponds to AZFa region. Deletion analysis revealed that deletion of UTY is associated DBY [79].

CANDIDATE GENES IN AZFB RESPONSIBLE FOR MALE INFERTILITY

RBMY

RBMY gene is a major gene in the AZFb region where it has six copies located on the Y chromosome [80]. RBMY1 codes for RNA-binding protein [81], which is a testis-specific splicing factor expressed in the nuclei of premature forms of spermatozoa [72]. In a study, it was observed that RBMY1 expression was reduced in azoospermic men [82].

PRY

The PRY genes are participating in the regulation of apoptosis which is an essential process to remove abnormal sperm from the population of spermatozoa [72]. If both the RBMY and PRY genes are removed, spermatogenesis is arrested completely [83], this observation indicates that these genes are involved in fertility in the AZFb region.

CANDIDATE GENES IN AZFC RESPONSIBLE FOR MALE INFERTILITY

DAZ

DAZ is the prime candidate gene in the AZFc region. Several genes other than DAZ have been mapped to the AZFc region, including chromodomain Y 1 (CDY1). In men with azoospermia, oligospermia found with partial deletion in DAZ [72].

CDY1

Two CDY1 genes are mapped in the AZFc region, one within the DAZ cluster and the other at the distal end. This finding is fascinating since at least one CDY1 copy is invariably absent in patients with DAZ deletion [84].

BPY2, PRY, and TTY2

These genes are also located in AZFc region. The function of these genes is unknown, but they show similar characteristics like they have multiple copies on the Y chromosome, they expressed only in the testes, and they are Y specific. Deletions in these candidate genes are found, in 2-10% of azoospermic or severely oligozoospermic men [85].

Gr/gr deletions

The classical AZFc deletion, which removes 3.5 Mb between the b2/b4 amplicons, is the most common type of deletion. The frequency and pathological significance of these partial deletions are not yet clear, although recently, a novel Y chromosome 1.6-Mb deletion is derived as "gr/gr" deletion, and it specifically observed in infertile men with multiple degrees of spermatogenic failure [86]. The gr/gr deletion removes two copies of DAZ and one copy of CDY1 also several other transcription units in AZFc region. Since it is observed that passes from generation to generations, the gr/gr deletion results in subfertility rather than complete infertility. The deletion observed with numerous Y haplotypes, which suggests that it is multiple independent recombination events. Another deletion, named b2/b3 or u3-gr/gr or g1/g3, which removes a similar quantity of AZFc genes, has also been identified.

CANDIDATE GENES IN AZFD RESPONSIBLE FOR MALE INFERTILITY

No candidate gene has been identified till now in AZFd region. However, recently it is observed that the deletion of DYS237 locus in AZFd region indicated that it has some important of genes which required for spermatogenesis [87]. Patients with microdeletions restricted to AZFd may present with mild oligozoospermia or even a normal sperm count associated with abnormal sperm morphology. STS markers like SY133, SY145, SY153, and SY152 are used for screening the AZFd region. Many variations in the junction of the euchromatic and heterochromatic regions have been deleted, which are reported from the STS marker (SY153) in AZFd.

OTHER GENES

TSPY

The TSPY gene is located on Yp, and it also has copies on Yq [74]. TSPY is expressed in the testis, and its protein has been detected in spermatogonia [88]. The TSPY gene can regulate the timing of spermatogenesis by signaling spermatogonia to enter meiosis [73]. Further investigation has to be done on TSPY to characterize its role in infertility because in a previous study it was seen that infertile men has variations in copy numbers of TSPY gene [89].

Autosomal DAZL gene

The DAZ gene has an autosomal homolog, i.e., DAZL which has location as 3p24. It is highly homologous to the DAZ gene about 83% of similarities in the coding region of the cDNA. Both DAZ and DAZL are the RNA-binding protein [90]. In one of the previous study, it is observed that 60% of idiopathic male infertility arises from autosomal recessive mutations [91]. A novel mutation, i.e., AG transition at nucleotide 386 in exon 3 of the DAZL gene, was identified in some infertile male patients. The mutation is located in the RNA-recognition motif (aa32-117) domain of the DAZL protein, and it will lead to change in Thr54 Ala (T54A) of DAZL protein [92]. There are, however, no reported instances of DAZL gene mutations among infertile men in India [93]. Whether DAZL plays a crucial role in spermatogenesis in humans also merits investigation.

X linked genes

Many X-linked genes are expressed in the testis [94] and are thought to be involved in gametogenesis. The AR gene is located on the Xq11.2-q12 and has four functional domains: the *N*-terminal transactivation domain, the DNA binding domain, the hinge region and the ligand binding domain [73]. It plays a role in meiosis and converts spermatocytes to spermatids [95]. In the recent study of infertile men, it is determined that approximately 2% of mutations are there in AR gene while the control one has none of them [96]. Mutations of the AR gene may also lead to androgen insensitivity syndrome [97].

TAF7L

The TAF7L gene has also been studied as a possible contributor to infertility in men. It is expressed in the testis and is related to the autosomal TAF7 gene, which is a transcription factor [88]. Transcription factor regulators show integral roles in spermatogenesis because they control the spatial and temporal factors which are important for accurate execution of the process [98-100].

TEX11

In a recent study of hemizygous *TEX11* mutations were a common cause of meiotic arrest; probably in the formation of crossovers during meiosis and azoospermia in infertile men. In a previous study, it was found that 99- kb hemizygous loss on chromosome Xq13.2 which involved three exons of TEX11. This mutation was identical in patients with azoospermia and shows a deletion of 79 amino acids within the meiosis-specific domain, SPO22. Furthermore, subsequent mutation screening showed five novel *TEX11* mutations as three splicing mutations, two missense mutations. These mutations are observed in 2.4% of azoospermia men [101].

Kallmann Syndrome Interval Gene1 (KAL1)

KAL1 is located on the Xq arm which is responsible for migration of GnRH neurons, and it encodes anosmin-1 which is a cell adhesion molecule [102]. Defect in the migration of the GnRH neurons cause KS: It is an inherited disorder which can cause infertility in males, and it has both X-linked and autosomal genetic factors. KS is an idiopathic HH (IHH) combined with anosmia or hyposmia. KAL1 gene deletions were found in KS patients [102,103].

MITOCHONDRIAL DNA AND MALE INFERTILITY

DNA polymerase gamma (POLG)

Sperm mitochondria play an essential role in functions of spermatozoa; therefore, genetic alterations to mitochondrial DNA may have correlation with normal fertilization. The important nuclear enzyme for mitochdrial polymerase activity is DNA POLG. The catalytic subunit of POLG is encoded by the POLG gene, which has been mapped to Yq15q25 and it includes a CAG repeat region [104]. Mutations in the POLG gene affect the proofreading activity of the enzyme and results in the mutation of the mitochondrial genome and subsequently affect the ATP production. The association of a large stretch of CAG repeat is related with male infertility. Various studies have observed an association between different polymorphisms, mutations or deletions in the mitochondrial genome and sperm dysfunction [105]. One of the previous studies identified that specific mtDNA haplogroups are associated with asthenozoospermia.

Mitochondrial DNA mutations in spermatozoa

Spermatozoa need a great deal of energy to support their rapid movement after ejaculation. Thus, random attacks on the naked mtDNA of sperms by reactive oxygen species (ROS) or free radicals causes oxidative damage or mutation in the mitochondrial genome with pathological findings and results in male infertility [106]. The midpiece of the human sperm consists of 70-80 mitochondria in number, and there is one copy of mtDNA in each of them. About 85% of sperm samples contain large-scale mtDNA deletions of variable sizes, and most of them have 2-7 deletions of mtDNA in sperm. Among the mitochondrial deletions observed, the common deletion is in 4977 bp. Researchers have to study whether the mtDNA deletions are localized in the same mitochondria or in different organelles of spermatozoa which are harboring mutated mtDNA molecules. In one of the study, it was observed that a novel 2-bp deletion (8195 and 8196 nt) in the COII gene, which may result in a truncated protein [107]. In a previous study, it was found that two common substitutions at 9055 and 11719 in males responsible for reducing the sperm motility [108]. It has been found that high levels of A3243G mutant in mtDNA correlate with asthenozoospermia. The important role of genetic aberrations in etiology of human male infertility is increasingly recognized in research papers. While much remains to be find out in this fast-moving field. Much considerable progress has been made in the clinical delineation of genetic factors of male infertility. Diagrammatic representation of human mitochondrial DNA is presented in Fig. 5. Gene mutation and STS markers studied on male infertility in different population is presented in Table 3.

NOVEL TECHNOLOGIES USED IN DIAGNOSIS OF MALE INFERTILITY

Global approaches to the study of male infertility are currently being developed, which provides highly effective methods to diagnose and treat infertility. One of the main problems that geneticists face when they are relating a genotype to a specific infertility phenotype is that diversity in genetic backgrounds of different ethnic groups. Incorporating techniques as genomics, proteomics, and metabolomics into the field of ART develops a more approximate solutions in infertility.

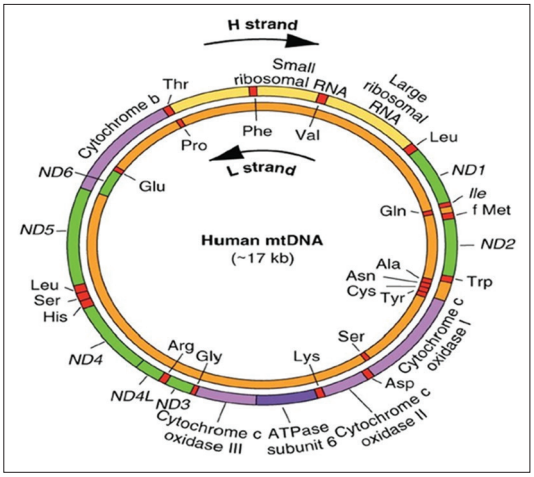

Fig. 5: Structure of Mitochondrial DNA [109]

Microarray

Microarrays are effective for identifying the gene expression profiles of infertile phenotypes [109]. Microarrays also examine the expression in spermatogenesis. Microarrays may be used to study the effect of hormones or growth factors on gene expression profiles. Microarray studies of gene expression produce various results to determine global gene expression patterns of RNA samples from the testis before it applied for clinical purpose.

Genomic methods

Genomic analysis is used to determine differentially transcribed genes [125]. Understanding of transcription regulation could help geneticists to discover the idea that how different expression patterns impact a patient's fertility [109]. Characterization of a gene expression signature for human spermatogenesis may be used as a baseline marker [97]. These markers also correlate between infertile phenotypes and mRNA expression [109].

Proteomics

Proteomics determine the protein expression profiles of fertile and infertile men. Proteins are recognized by 2D electrophoresis and mass spectrometry techniques, and the results are utilized for creation of maps in the proteome [126]. Spermatozoa are novel for the study of protein expression because they do not have active transcription or translation. Further research in this field can continue after, the sperm proteome is fully defined and the factors of seminal plasma are identified. Recognization of protein biomarkers for male infertility will allow for unbiased comparison between fertile and infertile males, and it will also give us idea about pathophysiology of the diseases [127]. An advantageous characteristic of genomic and proteomic technology is that the results can be confirmed using other techniques such as Western blots, flow cytometry, and PCR.

Metabolomics

Metabolomics is used to measure the expression of metabolites, small biomarkers which indicate the functionality of a cell, and characterize them for certain diseases or physiological states [128,129] Mass spectroscopy, nuclear magnetic resonance spectroscopy, and chromatography methods may be used to create profiles of metabolites. Pathway or cluster analysis is used to define subsets of metabolites that used to quantitatively characterize patients for diagnosis [130]. After identifying differences in the expression of metabolites in infertile

Table 3: Gene mutation and polymorphism studied on male infertility in different population

Clinical syndrome related to human male infertlity	Responsible genes	Location/variations found	Ethnicity	References
Sertoli cell only syndrome	DBY (DDX3Y)	Yq11.21/reduced expression	German	[72]
Azoospermia, oligospermia or oligoasthenozoospermia	USP9Y	Yq11.21/deletion	Italian	[75]
Azoospermia	RBMY	Yq11.223/deletion	Australian	[110]
Abnormalities in sperms	PRY	Yq11.223/deletion	German	[72]
Azoospermia, oligospermia	DAZ	Yq11.223/partial deletion	Italian	[84]
Azoospermia, oligospermia	CDY	Yq11.23/deletion	Italian	[84]
Subfertility	DAZ, CDY1	Yq11.223/Gr/gr deletion	Spanish	[86]
Oligozoospermia	Unknown	DYS237/deletion	Turkish	[87]
Abnormalities in sperms	TSPY	Yp11.2/variation in number of copies	Czechs	[89]
Azoospermia	TEX11	Xq13.1/deletion, missense mutations, splicing mutations	German	[101]
Spermatogenic failure	TAF7L	Xq22.1/deletion, point mutation	Caucasian	[100]
Kallman syndrome	KAL1	Xp22.31/deletion	American	[102]
Kallman syndrome	FGFR1	8p11.23/deletion	American	[102]
Oligoasthenoteratozoo spermia	POLG	Yq15/deletion, long stretch of CAG repeats	British	[105]
Asthenozoospermia	COII	Mt DNA/deletion	Indian	[108]
CBAVD	CFTR	7q31.2/deletion	Greek	[111]
Asthenozoospermia	SHBG	17p13.1/deletion	Greek	[112]
Oligozoospermia, cryptorchidism	ESR1	6q25.1/deletion, AGATA haplotype	Japanese	[113]
Spermatogenic failure	FSHR	2p16.3/partial deletion	Finnish	[114]
Spermatogenic failure	MTHFR	1p36.22/point mutation	African, South East Asian, Indian	[115-117]
Cryptorchidism	INSL3	19p13.11/deletion	Italian	[118]
Azoospermia	DAZL	3p24.3/point mutation	Taiwanese	[92]
Unknown (In mouse - severe spermatogenic impairment)	UTY	Yq11.221/deletion	Italian	[79]
Azoospermia	VCY2	Yq11.223/deletion	Chinese	[119]
Immature spermatocyte	HSFY	Yq11.222/deletion	French	[120]
Unknown	KDM5D	Yq11.222/deletion	Portuguese	[121]
Unknown	RPS4Y2	Yq11.223/deletion	Indian	[122]
Spermatogenic failure	SPGFY1	Yq11/microdeletion	German	[69]
Spermatogenic failure, idiopathic azoospermia	SPGFY2	Yq11.21/deletion	Italian	[84]
Unknown	TB4Y	Yq11.221/deletion	American	[123]
Unknown	XKRY	Yq11.221/deletion	Italian	[124]
Unknown	EIF1AY	Yq11.223/deletion	Indian	[125]

DDX3Y: DEAD/H Box 3; Y-Linked, USP9Y: Ubiqutin - specific protease 9; Y chromosome, RBMY: RNA - binding motif protein; Y CHROMOSOME, PRY: PTPBL related gene on Y, DAZ: Deleted in azoospermia, CDY: Chromodomain protein; Y chromosome, TSPY: Testis specific protein; Y linked, TEX11: Tesis Expressed Gene 11, TAF7L: TATA box binding protein associated factor, KAL1: Kallmann syndrome interval gene1, FGFR1: Fibroblast growth factor receptor 1, POLG: Polymerase DNA gamma, COII: Cytocrome C oxidase subunit II, CFTR: Cystic fibrosis transmembrane conductance regulator, SHBG: Sex hormone binding globulin, ESR1: Estrogen receptor 1, FSHR: Follicle stimulating hormone receptor, MTHFR: 5,10 Methenetetrahydrofolate reductase, INSL3: Insulin like 3, DAZL: Deleted in azoospermia like, UTY: Ubiquitously transcribed tetratrichopeptide repeat gene on Y chromosome, VCY2: Variably charged Y chromosome 2, HSFY: Heat shock transcription factor; Y linked, KDM5D: Lysine specific demethylase 5D, RPS4Y2: Ribosomal protein S4; Y linked 2, SPGFY: Spermatogenic failure1, TB4Y: Thymosin beta chromosome 4; Y chromosome, XKRY: XK related protein; Y chromosome, EIF1AY: Eukaryotic translation initiation factor 1A; Y linked

phenotypes, new methods can be developed which are inexpensive and noninvasive in diagnostic field. Furthermore, metabolomics used to identify biomarkers for OS, which are giving signals to examine the semen quality [128].

ICSI

Males with Yq microdeletions are mostly infertile, but many can still fertile through ICSI using the few viable sperms or mature spermatids isolated directly from the epididymis or testes. This sperm recovery technique helps the obstructive and non-obstructive azoospermic patients to achieve genetic fatherhood. However, by this technique transmission of microdeletions to sons has recently been described [131]. This observation implies that in the future, there will be a much greater overlap between reproductive medicine and genetics, and these two fields correlate very closely to treat patients with infertility in the best possible way.

CONCLUSION

Genes required for spermatogenesis has may mutations and deletions, which results in male infertility. Previous research findings suggest that genetic and some environmental factors are responsible for infertility of men. The molecular techniques used in ART have provided great insight into the genetics of male infertility. Through the efforts of researchers, it is possible to find relation between genetic mutations, environmental risk factors, and ethnic background to specific infertile phenotypes in men. Using this knowledge background, clinicians will be able to treat infertile males efficiently. Although much work still to be completed to fully determine the correlation between genotype and phenotype of male infertility.

ACKNOWLEDGMENTS

Authors would like to thank the management of VIT University for providing the facilities to carry out this work.

REFERENCES

1. Poongothai J, Gopenath TS, Manonayaki S. Genetics of human male infertility. Singapore Med J 2009;50(4):336-47.
2. Zorgniotti AW, Sealfon AI, Toth A. Further clinical experience with testis hypothermia for infertility due to poor semen. Urology 1982;19(6):636-40.
3. Carosi L, Calabrò F. Fertility in couples working in noisy factories. Folia Med (Napoli) 1968;51(4):264-8.
4. Sandeman TF. The effects of x irradiation on male human fertility. Br J Radiol 1966;39(468):901-7.

5. Lancranjan I, Maicanescu M, Rafaila E, Klepsch I, Popescu HI. Gonadic function in workmen with long-term exposure to microwaves. Health Phys 1975;29(3):381-3.
6. Kenkel S, Rolf C, Nieschlag E. Occupational risks for male fertility: An analysis of patients attending a tertiary referral centre. Int J Androl 2001;24(6):318-26.
7. Levine RJ, Mathew RM, Chenault CB, Brown MH, Hurtt ME, Bentley KS, *et al*. Differences in the quality of semen in outdoor workers during summer and winter. N Engl J Med 1990;323(1):12-6.
8. Thonneau P, Ducot B, Bujan L, Mieusset R, Spira A. Heat exposure as a hazard to male fertility. Lancet 1996;347(8995):204-5.
9. Selevan SG, Borkovec L, Slott VL, Zudová Z, Rubes J, Evenson DP, *et al.* Semen quality and reproductive health of young Czech men exposed to seasonal air pollution. Environ Health Perspect 2000;108(9):887-94.
10. Jørgensen N, Andersen AG, Eustache F, Irvine DS, Suominen J, Petersen JH, *et al.* Regional differences in semen quality in Europe. Hum Reprod 2001;16(5):1012-9.
11. Wong WY, Zielhuis GA, Thomas CM, Merkus HM, Steegers-Theunissen RP. New evidence of the influence of exogenous and endogenous factors on sperm count in man. Eur J Obstet Gynecol Reprod Biol 2003;110(1):49-54.
12. De Gennaro L, Balistreri S, Lenzi A, Lombardo F, Ferrara M, Gandini L. Psychosocial factors discriminate oligozoospermic from normozoospermic men. Fertil Steril 2003;79 Suppl 3:1571-6.
13. Muthusami KR, Chinnaswamy P. Effect of chronic alcoholism on male fertility hormones and semen quality. Fertil Steril 2005;84(4):919-24.
14. Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. Reprod Biol Endocrinol 2015;13:37.
15. Stewart DI. Epidemiology and aetiology of male infertility. ESHRE (European Society of Human Reproduction and Embryology) Andrology Special Interest Group. Hum Reprod 2015;13:33-44.
16. Winters BR, Thomas JW. The epidemiology of male infertility. Reprod Biol Endocrinol 2014;41:195-204.
17. Harton GL, Tempest HG. Chromosomal disorders and male infertility. Asian J Androl 2012;14(1):32-9.
18. Seshagiri PB. Molecular insights into the causes of male infertility. J Biosci 2001;26 4 Suppl:429-35.
19. Peterson CM. Human reproduction: Clinical, pathologic and pharmacologic correlations. In: Hum Rep Seminars. Available from: Library.med.utah.edu/kw/human_reprod/seminars/seminar2B.html. [Last accessed on 2006 Jan 04].
20. O'Flynn O'Brien KL, Varghese AC, Agarwal A. The genetic causes of male factor infertility: A review. Fertil Steril 2010;93(1):1-12.
21. European Association of Urology. Guidelines on male infertility. Updated April 2010. Available from: http://www.uroweb.org/gls/pdf/14_Male_Infertility%202010.pdf. [Last accessed on 2011 Nov 29].
22. Oldereid NB, Rui H, Purvis K. Life styles of men in barren couples and their relationship to sperm quality. Int J Fertil 1992;37(6):343-9.
23. Effendy I, Krause W. Environmental risk factors in the history of male patients of an infertility clinic. Andrologia 1987;19:262-5.
24. European Association of Urology Guidelines on Male Infertility: The 2012 Update. Arnhem, Netherlands: European Association of Urology. Available from: http://www.uroweb.org/guidelines/online-guidelines. [Last accessed on 2012 Jul 20].
25. McPhee SJ, Maxine AP, Michael WR. Current Medical Diagnosis and Treatment 2012. 51st ed. New York, N.Y.: The McGraw-Hill Companies; 2012. Available from: http://www.accessmedicine.com/resourceTOC.aspx?resourceID=1. [Last accessed on 2012 Jul 20].
26. Swerdloff RF, Christina W. Causes of male infertility. Available from: http://www.uptodate.com/home/index. [Last accessed on 2012 Jul 19].
27. Patel ZP, Niederberger CS. Male factor assessment in infertility. Med Clin North Am 2011;95(1):223-34.
28. The Optimal Evaluation of the Infertile Male: AUA Best Practice Statement. Linthicum, Md.: American Urological Association Education and Research, Inc. Available from: http://www.guideline.gov/content.aspx?id=23921&search=male+infertility. [Last accessed on 2012 Jul 19].
29. Loughlin KR. Changes in male fertility in the last two decades. Urol Clin North Am 2012;39(1):33-6.
30. Asplund C, Barkdull T, Weiss BD. Genitourinary problems in bicyclists. Curr Sports Med Rep 2007;6(5):333-9.
31. Frey KA. Male reproductive health and infertility. Prim Care 2010;37(3):643-52, x.
32. Showell MG, Brown J, Yazdani A, Stankiewicz MT, Hart RJ. Antioxidants for male subfertility. Cochrane Database of Syst Rev. Available from: http://www.onlinelibrary.wiley.com/doi/10.1002/14651858.CD007411.pub2/abstract. [Last accessed 2012 Jul 23].
33. Patel SR, Sigman M. Antioxidant therapy in male infertility. Urol Clin North Am 2008;35(2):319-30, x.
34. De Celis R, Feria-Velasco A, González-Unzaga M, Torres-Calleja J, Pedrón-Nuevo N. Semen quality of workers occupationally exposed to hydrocarbons. Fertil Steril 2000;73(2):221-8.
35. Swan SH. Semen quality in fertile US men in relation to geographical area and pesticide exposure. Int J Androl 2006;29(1):62-8.
36. El-Helaly M, Awadalla N, Mansour M, El-Biomy Y. Workplace exposures and male infertility - a case-control study. Int J Occup Med Environ Health 2010;23(4):331-8.
37. Murphy LE, Mills JL, Molloy AM, Qian C, Carter TC, Strevens H, *et al.* Folate and vitamin B12 in idiopathic male infertility. Asian J Androl 2011;13(6):856-61.
38. Lenzi A, Lombardo F, Sgrò P, Salacone P, Caponecchia L, Dondero F, *et al.* Use of carnitine therapy in selected cases of male factor infertility: A double-blind crossover trial. Fertil Steril 2003;79(2):292-300.
39. Ross C, Morriss A, Khairy M, Khalaf Y, Braude P, Coomarasamy A, *et al.* A systematic review of the effect of oral antioxidants on male infertility. Reprod Biomed Online 2010;20(6):711-23.
40. Ménézo YJ, Hazout A, Panteix G, Robert F, Rollet J, Cohen-Bacrie P, *et al.* Antioxidants to reduce sperm DNA fragmentation: An unexpected adverse effect. Reprod Biomed Online 2007;14(4):418-21.
41. Thonneau P, Marchand S, Tallec A, Ferial ML, Ducot B, Lansac J, *et al.* Incidence and main causes of infertility in a resident population (1,850,000) of three French regions (1988-1989). Hum Reprod 1991;6(6):811-6.
42. Jarow JP, Espeland MA, Lipshultz LI. Evaluation of the azoospermic patient. J Urol 1989;142(1):62-5.
43. Jarvi K, Lo K, Fischer A, Grantmyre J, Zini A, Chow V, *et al.* CUA Guideline: The workup of azoospermic males. Can Urol Assoc J 2010;4(3):163-7.
44. Practice Committee of American Society for Reproductive Medicine in collaboration with Society for male reproduction and urology. The management of infertility due to obstructive azoospermia. Fertil Steril 2008;90 5 Suppl: S121-4.
45. Kumar R. Medical management of non-obstructive azoospermia. Clinics (Sao Paulo) 2013;68 Suppl 1:75-9.
46. Reijo R, Alagappan RK, Patrizio P, Page DC. Severe oligozoospermia resulting from deletions of azoospermia factor gene on Y chromosome. Lancet 1996;347(9011):1290-3.
47. Comhaire FH, de Kretser D, Farley TM, Rowe PJ. WHO taskforce on the diagnosis and treatment of infertility. In: Skakkebaek NE, editor. Towards More Objectivity in Diagnosis and Management of Male Infertility. Geneva: WHO; 1987. p. 1-33.
48. David LH. Semen Analysis-Methods of Hematology. Westerville, Ohio- 43082: Sweet Haven Publishing Services; 2004.
49. Shamsi MB, Kumar K, Dada R. Genetic and epigenetic factors: Role in male infertility. Indian J Urol 2011;27(1):110-20.
50. Smith-Whitley K. Reproductive issues in sickle cell disease. Blood 2014;124(24):3538-43.
51. Zhang Y, Malekpour M, Al-Madani N, Kahrizi K, Zanganeh M, Lohr NJ, *et al.* Sensorineural deafness and male infertility: A contiguous gene deletion syndrome. J Med Genet 2007;44(4):233-40.
52. Hargreave TB, McGowan B, Harvey J, McParland M, Elton RA. Is a male infertility clinic of any use? Br J Urol 1986;58(2):188-93.
53. Gonçalves J, Lavinha J. Y chromosome and male infertility. Acta Med Port 1998;11(4):365-72.
54. Edwards RG, Bishop CE. On the origin and frequency of Y chromosome deletions responsible for severe male infertility. Mol Hum Reprod 1997;3(7):549-54.
55. McLachlan RI, Mallidis C, Ma K, Bhasin S, de Kretser DM. Genetic disorders and spermatogenesis. Reprod Fertil Dev 1998;10(1):97-104.
56. Stouffs K, Lissens W, Tournaye H, Haentjens P. What about gr/gr deletions and male infertility? Systematic review and meta-analysis. Hum Reprod Update 2011;17(2):197-209.
57. Abilash VG, Saraswathy R, Marimuthu KM. The frequency of Y chromosome microdeletions in infertile men from Chennai, a South East Indian population and the effect of smoking, drinking alcohol and chemical exposure on their frequencies. Int J Genet Mol Biol 2010;2:147-57.
58. Gazvani R, Lewis-Jones DI. Cystic fibrosis mutation screening before assisted reproduction. Int J Androl 2004;27(1):1-4.
59. Shamsi MB, Kumar R, Bhatt A, Bamezai RN, Kumar R, Gupta NP, *et al.* Mitochondrial DNA Mutations in etiopathogenesis of male infertility. Indian J Urol 2008;24(2):150-4.

60. Spiropoulos J, Turnbull DM, Chinnery PF. Can mitochondrial DNA mutations cause sperm dysfunction? Mol Hum Reprod 2002;8(8):719-21.
61. Singh AR, Vrtel R, Vodicka R, Dhaifalah I, Konvalinka D, Janikova M. Y chromosome and Male Infertility. Int J Hum Genet 2005;5:225-35.
62. Ali S, Hasnain SE. Molecular dissection of the human Y-chromosome. Gene 2002;283(1-2):1-10.
63. Tilford CA, Kuroda-Kawaguchi T, Skaletsky H, Rozen S, Brown LG, Rosenberg M, *et al.* A physical map of the human Y chromosome. Nature 2001;409(6822):943-5.
64. Foote S, Vollrath D, Hilton A, Page DC. The human Y chromosome: Overlapping DNA clones spanning the euchromatic region. Science 1992;258(5079):60-6.
65. Ali S, Hasnain SE. Genomics of the human Y-chromosome 1. Association with male infertility. Gene 2003;321:25-37.
66. Nudell DM, Pagani R, Larry IL. Indication for genetic evaluation of men in a reproductive medicine program. Braz J Urol 2001;27:105-19.
67. Chandley AC, Cooke HJ. Human male fertility – Y-linked genes and spermatogenesis. Hum Mol Genet 1994;3:1449-52.
68. Liow SL, Ghadessy FJ, Ng SC, Yong EL. Y chromosome microdeletions, in azoospermic or near-azoospermic subjects, are located in the AZFc (DAZ) subregion. Mol Hum Reprod 1998;4(8):763-8.
69. Vogt PH, Edelmann A, Kirsch S, Henegariu O, Hirschmann P, Kiesewetter F, *et al.* Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. Hum Mol Genet 1996;5(7):933-43.
70. Vogt PH, Affara N, Davey P, Hammer M, Jobling MA, Lau YF, *et al.* Report of the Third International Workshop on Y chromosome mapping 1997. Heidelberg, Germany, April 13-16, 1997. Cytogenet Cell Genet 1997;79(1-2):1-20.
71. Dada R, Gupta NP, Kucheria K. Yq microdeletions – Azoospermia factor candidate genes and spermatogenic arrest. J Biomol Tech 2004;15(3):176-83.
72. Vogt PH. Azoospermia factor (AZF) in Yq11: Towards a molecular understanding of its function for human male fertility and spermatogenesis. Reprod Biomed Online 2005;10(1):81-93.
73. Nuti F, Krausz C. Gene polymorphisms/mutations relevant to abnormal spermatogenesis. Reprod Biomed Online 2008;16(4):504-13.
74. Lardone MC, Parodi DA, Valdevenito R, Ebensperger M, Piottante A, Madariaga M, *et al.* Quantification of DDX3Y, RBMY1, DAZ and TSPY mRNAs in testes of patients with severe impairment of spermatogenesis. Mol Hum Reprod 2007;13(10):705-12.
75. Tyler-Smith C. An evolutionary perspective on Y-chromosomal variation and male infertility. Int J Androl 2008;31(4):376-82.
76. Galan JJ, Guarducci E, Nuti F, Gonzalez A, Ruiz M, Ruiz A, *et al.* Molecular analysis of estrogen receptor alpha gene AGATA haplotype and SNP12 in European populations: Potential protective effect for cryptorchidism and lack of association with male infertility. Hum Reprod 2007;22(2):444-9.
77. Brown GM, Furlong RA, Sargent CA, Erickson RP, Longepied G, Mitchell M, *et al.* Characterisation of the coding sequence and fine mapping of the human DFFRY gene and comparative expression analysis and mapping to the Sxrb interval of the mouse Y chromosome of the Dffry gene. Hum Mol Genet 1998;7(1):97-107.
78. Krausz C, Degl'Innocenti S, Nuti F, Morelli A, Felici F, Sansone M, *et al.* Natural transmission of USP9Y gene mutations: A new perspective on the role of AZFa genes in male fertility. Hum Mol Genet 2006;15(18):2673-81.
79. Foresta C, Ferlin A, Moro E. Deletion and expression analysis of AZFa genes on the human Y chromosome revealed a major role for DBY in male infertility. Hum Mol Genet 2000;9(8):1161-9.
80. Skaletsky H, Kuroda-Kawaguchi T, Minx PJ, Cordum HS, Hillier L, Brown LG, *et al.* The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. Nature 2003;423(6942):825-37.
81. Elliott DJ. The role of potential splicing factors including RBMY, RBMX, hnRNPG-T and STAR proteins in spermatogenesis. Int J Androl 2004;27(6):328-34.
82. Lavery R, Glennon M, Houghton J, Nolan A, Egan D, Maher M. Investigation of DAZ and RBMY1 gene expression in human testis by quantitative real-time PCR. Arch Androl 2007;53(2):71-3.
83. Vogt PH. Human chromosome deletions in Yq11, AZF candidate genes and male infertility: History and update. Mol Hum Reprod 1998;4(8):739-44.
84. Foresta C, Moro E, Ferlin A. Y chromosome microdeletions and alterations of spermatogenesis. Endocr Rev 2001;22(2):226-39.
85. Tiepolo L, Zuffardi O. Localization of factors controlling spermatogenesis in the nonfluorescent portion of the human Y chromosome long arm. Hum Genet 1976;34(2):119-24.
86. de Llanos M, Ballescà JL, Gázquez C, Margarit E, Oliva R. High frequency of gr/gr chromosome Y deletions in consecutive oligospermic ICSI candidates. Hum Reprod 2005;20(1):216-20.
87. Müslümanoglu MH, Turgut M, Cilingir O, Can C, Ozyürek Y, Artan S. Role of the AZFd locus in spermatogenesis. Fertil Steril 2005;84(2):519-22.
88. Schnieders F, Dörk T, Arnemann J, Vogel T, Werner M, Schmidtke J. Testis-specific protein, Y-encoded (TSPY) expression in testicular tissues. Hum Mol Genet 1996;5(11):1801-7.
89. Vodicka R, Vrtel R, Dusek L, Singh AR, Krizova K, Svacinova V, *et al.* TSPY gene copy number as a potential new risk factor for male infertility. Reprod Biomed Online 2007;14(5):579-87.
90. Tsui S, Dai T, Roettger S, Schempp W, Salido EC, Yen PH. Identification of two novel proteins that interact with germ-cell-specific RNA-binding proteins DAZ and DAZL1. Genomics 2000;65(3):266-73.
91. Lilford R, Jones AM, Bishop DT, Thornton J, Mueller R. Case-control study of whether subfertility in men is familial. BMJ 1994;309(6954):570-3.
92. Teng YN, Lin YM, Lin YH, Tsao SY, Hsu CC, Lin SJ, *et al.* Association of a single-nucleotide polymorphism of the deleted-in-azoospermia-like gene with susceptibility to spermatogenic failure. J Clin Endocrinol Metab 2002;87(11):5258-64.
93. Thangaraj K, Deepa SR, Pavani K, Gupta NJ, Reddy P, Reddy AG, *et al.* A to G transitions at 260, 386 and 437 in DAZL gene are not associated with spermatogenic failure in Indian population. Int J Androl 2006;29(5):510-14.
94. Wang PJ, McCarrey JR, Yang F, Page DC. An abundance of X-linked genes expressed in spermatogonia. Nat Genet 2001;27(4):422-6.
95. De Gendt K, Swinnen JV, Saunders PT, Schoonjans L, Dewerchin M, Devos A, *et al.* A Sertoli cell-selective knockout of the androgen receptor causes spermatogenic arrest in meiosis. Proc Natl Acad Sci U S A 2004;101(5):1327-32.
96. Ferlin A, Vinanzi C, Garolla A, Selice R, Zuccarello D, Cazzadore C, *et al.* Male infertility and androgen receptor gene mutations: Clinical features and identification of seven novel mutations. Clin Endocrinol (Oxf) 2006;65(5):606-10.
97. Ferlin A, Raicu F, Gatta V, Zuccarello D, Palka G, Foresta C. Male infertility: Role of genetic background. Reprod Biomed Online 2007;14(6):734-45.
98. Sassone-Corsi P. Transcriptional checkpoints determining the fate of male germ cells. Cell 1997;88(2):163-6.
99. Kimmins S, Kotaja N, Davidson I, Sassone-Corsi P. Testis-specific transcription mechanisms promoting male germ-cell differentiation. Reproduction 2004;128(1):5-12.
100. Akinloye O, Gromoll J, Callies C, Nieschlag E, Simoni M. Mutation analysis of the X-chromosome linked, testis-specific TAF7L gene in spermatogenic failure. Andrologia 2007;39(5):190-5.
101. Yatsenko AN, Georgiadis AP, Röpke A, Berman AJ, Jaffe T, Olszewska M, *et al.* X-linked TEX11 mutations, meiotic arrest, and azoospermia in infertile men. N Engl J Med 2015;372(22):2097-107.
102. Bhagavath B, Layman LC. The genetics of hypogonadotropic hypogonadism. Semin Reprod Med 2007;25(4):272-86.
103. Maglott D, Ostell J, Pruitt KD, Tatusova T. Entrez Gene: Gene-centered information at NCBI. Nucleic Acids Res 2005;33:D54-8.
104. Ropp PA, Copeland WC. Cloning and characterization of the human mitochondrial DNA polymerase, DNA polymerase gamma. Genomics 1996;36(3):449-58.
105. St John JC, Jokhi RP, Barratt CL. Men with oligoasthenoteratozoospermia harbour higher numbers of multiple mitochondrial DNA deletions in their spermatozoa, but individual deletions are not indicative of overall aetiology. Mol Hum Reprod 2001;7(1):103-11.
106. Wei YH, Kao SH. Mitochondrial DNA mutation and depletion are associated with decline of fertility and motility of human sperm. Zool Stud 2000;39:1-12.
107. Thangaraj K, Joshi MB, Reddy AG, Rasalkar AA, Singh L. Sperm mitochondrial mutations as a cause of low sperm motility. J Androl 2003;24(3):388-92.
108. Ellis PJ, Furlong RA, Conner SJ, Kirkman-Brown J, Afnan M, Barratt C, *et al.* Coordinated transcriptional regulation patterns associated with infertility phenotypes in men. J Med Genet 2007;44(8):498-508.
109. Kumar DP, Sangeetha N. Mitochondrial DNA mutations and male infertility. Indian J Hum Genet 2009;15(3):93-7.
110. Huynh T, Mollard R, Trounson A. Selected genetic factors associated with male infertility. Hum Reprod Update 2002;8(2):183-98.
111. Georgiou I, Syrrou M, Pardalidis N, Karakitsios K, Mantzavinos T,

Giotitsas N, *et al.* Genetic and epigenetic risks of intracytoplasmic sperm injection method. Asian J Androl 2006;8(6):643-73.
112. Lazaros L, Xita N, Kaponis A, Zikopoulos K, Sofikitis N, Georgiou I. Evidence for association of sex hormone-binding globulin and androgen receptor genes with semen quality. Andrologia 2008;40(3):186-91.
113. Yoshida R, Fukami M, Sasagawa I, Hasegawa T, Kamatani N, Ogata T. Association of cryptorchidism with a specific haplotype of the estrogen receptor alpha gene: Implication for the susceptibility to estrogenic environmental endocrine disruptors. J Clin Endocrinol Metab 2005;90(8):4716-21.
114. Tapanainen JS, Aittomäki K, Min J, Vaskivuo T, Huhtaniemi IT. Men homozygous for an inactivating mutation of the follicle-stimulating hormone (FSH) receptor gene present variable suppression of spermatogenesis and fertility. Nat Genet 1997;15(2):205-6.
115. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, *et al.* A candidate genetic risk factor for vascular disease: A common mutation in methylenetetrahydrofolate reductase. Nat Genet 1995;10(1):111-3.
116. Park JH, Lee HC, Jeong YM, Chung TG, Kim HJ, Kim NK, *et al.* MTHFR C677T polymorphism associates with unexplained infertile male factors. J Assist Reprod Genet 2005;22(9-10):361-8.
117. Singh K, Singh SK, Sah R, Singh I, Raman R. Mutation C677T in the methylenetetrahydrofolate reductase gene is associated with male infertility in an Indian population. Int J Androl 2005;28(2):115-9.
118. Ferlin A, Foresta C. The INSL3-LGR8 hormonal system in humans: Testicular descent, cryptorchidism and testicular functions. Curr Med Chem 2005;15:421-30.
119. Tse JY, Wong EY, Cheung AN, O WS, Tam PC, Yeung WS. Specific expression of VCY2 in human male germ cells and its involvement in the pathogenesis of male infertility. Biol Reprod 2003;69(3):746-51.
120. Kichine E, Rozé V, Di Cristofaro J, Taulier D, Navarro A, Streichemberger E, *et al.* HSFY genes and the P4 palindrome in the AZFb interval of the human Y chromosome are not required for spermatocyte maturation. Hum Reprod 2012;27(2):615-24.
121. Navarro-Costa P, Plancha CE, Gonçalves J. Genetic dissection of the AZF regions of the human Y chromosome: Thriller or filler for male (in)fertility? J Biomed Biotechnol 2010;2010:936569.
122. Vineeth VS, Suttur SM. A journey on Y chromosomal genes and male infertility. Int J Hum Genet 2011;11:203-15.
123. Kostiner DR, Turek PJ, Reijo RA. Male infertility: Analysis of the markers and genes on the human Y chromosome. Hum Reprod 1998;13(11):3032-8.
124. Ferlin A, Moro E, Rossi A, Dallapiccola B, Foresta C. The human Y chromosome's azoospermia factor b (AZFb) region: Sequence, structure, and deletion analysis in infertile men. J Med Genet 2003;40(1):18-24.
125. Shima JE, McLean DJ, McCarrey JR, Griswold MD. The murine testicular transcriptome: Characterizing gene expression in the testis during the progression of spermatogenesis. Biol Reprod 2004;71(1):319-30.
126. Martínez-Heredia J, de Mateo S, Vidal-Taboada JM, Ballescà JL, Oliva R. Identification of proteomic differences in asthenozoospermic sperm samples. Hum Reprod 2008;23(4):783-91.
127. Barratt CL. The human sperm proteome: The potential for new biomarkers of male fertility and a transformation in our understanding of the spermatozoon as a machine: Commentary on the article 'Identification of proteomic differences in asthenozoospermic sperm samples' by Martinez *et al.* Hum Reprod 2008;23(6):1240-1.
128. Deepinder F, Chowdary HT, Agarwal A. Role of metabolomic analysis of biomarkers in the management of male infertility. Expert Rev Mol Diagn 2007;7(4):351-8.
129. Hollywood K, Brison DR, Goodacre R. Metabolomics: Current technologies and future trends. Proteomics 2006;6(17):4716-23.
130. German JB, Hammock BD, Watkins SM. Metabolomics: Building on a century of biochemistry to guide human health. Metabolomics 2005;1(1):3-9.
131. Stouffs K, Lissens W, Tournaye H, Van Steirteghem A, Liebaers I. The choice and outcome of the fertility treatment of 38 couples in whom the male partner has a Yq microdeletion. Hum Reprod 2005;20(7):1887-96.